

Application Note

Whatman FTA®

Amplification of Human Genomic DNA from Blood on FTA with GenomiPhi™

Techniques and Technical Insight to Optimize Use of Whatman Products

Whatman FTA is an excellent DNA storage and preparation medium for samples to be amplified by strand displacement amplification.

Whatman FTA® products are powerful tools that simplify the collection, shipment, storage and purification of nucleic acids from a wide variety of sources. Animal, plant or microbe cellular samples are applied directly onto an FTA Card, activating chemicals in the card that lyse the cells, inactivate proteins and immobilize the genomic nucleic acids. Infectious organisms are inactivated in the process. After drying, the nucleic acids are protected from enzymatic, microbial, oxidative and free-radical degradation. The samples can be stored for years at room temperature before analysis (14 years to date for blood on FTA).

For analysis or use of the stored DNA a small disk is cut from the sample area on FTA. Washing with FTA Purification Reagent removes contaminants. DNA immobilized in the washed disk can then be amplified by polymerase chain reaction (PCR), cut by restriction endonucleases or used in other ways. For some applications, however, larger amounts of DNA are needed than the disk—or even the whole original sample—contains. They may also require more sections of the sequence than even multiplex PCR can supply. These applications have in the past required isolation of DNA from larger samples. Whole genome amplification (WGA) has been developed to supply essentially unlimited quantities of DNA from small samples. This application note describes the use of strand displacement amplification, a popular WGA method, to generate micrograms of DNA from a small sample of human blood cell DNA preserved on FTA.

The GenomiPhi™ DNA Amplification Kit from Amersham Biosciences uses Phi29 DNA polymerase and a multiple strand displacement mechanism to amplify small initial amounts of linear template DNA. It produces microgram quantities of high molecular weight copies that represent the whole template sequence. A small input sample preserved on FTA can provide enough amplified DNA for multiple SNP analyses and other downstream applications. (See www.amershambiosciences.com or www.genomiphi.com for details.) This application note shows an example of GenomiPhi amplification of human genomic DNA stored and purified on FTA.

Methods

Following standard FTA protocols, fresh human blood from a finger-stick was spotted onto an FTA Card and allowed to dry. The sample was then stored in the dark at room temperature for 5 weeks. Disks of 1.2 or 2.0 mm diameter were cut from the bloodstained areas with a Harris Micro Punch. Control disks were cut from unused areas of the card. All disks were washed three times for 5 minutes with 200 μ L FTA Purification Reagent and twice for 5 minutes with 200 μ L TE⁻¹ (10 mM Tris, 0.1 mM EDTA, pH 8).



Amplification followed the standard GenomiPhi protocol. Each washed disk was added to 9 μ L GenomiPhi Sample Buffer. Control disks received 20 ng purified human genomic DNA before amplification. Samples were incubated 3 minutes at 95°C and 3 minutes at 4°C. 10 μ L GenomiPhi Reaction Buffer containing 1 μ L polymerase was added to each tube. The tubes were briefly mixed by finger vortexing and incubated overnight (about 16 hours) at 30°C, then 10 minutes at 65°C and were finally stored at 4°C. A 4 μ L sample was removed from each tube for electrophoresis in 0.6% agarose containing ethidium bromide.

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Recommended Protocol

- 1) Preserve sample normally on FTA. Store in a dark, dry place at room temperature.
- 2) Punch disks (1.2 mm for blood) and transfer to tubes.
- 3) Wash disks 3 x 5 minutes in 200 μ L FTA Reagent.
- 4) Rinse disks 2 x 5 minutes in 200 μ L TE⁻¹. Disks should be colorless.
- 5) Dry disks 1 hour at room temperature (optional).
- 6) Add 10 μ L (or 9 μ L for wet disks) GenomiPhi sample buffer.
- 7) Incubate 3 minutes at 95°C and chill on ice.
- 8) Centrifuge briefly if necessary to collect condensate.
- 9) Add 10 μ L GenomiPhi Reaction Mix containing 1 μ L Phi29 polymerase and mix.
- 10) Incubate overnight (or about 16 hours) at 30°C.
- 11) Incubate 10 minutes at 65°C and store at 4°C.

Results

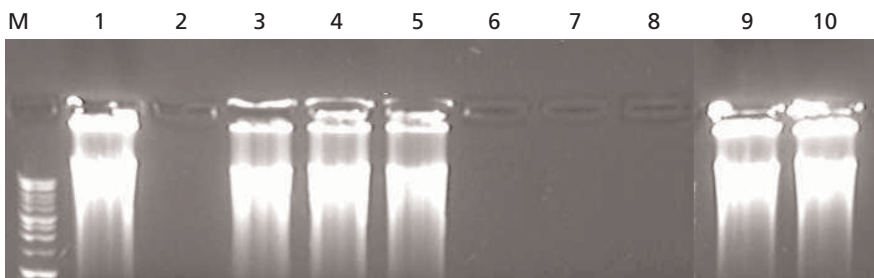
The figure shows successful GenomiPhi amplification of human genomic DNA immobilized on 1.2 mm disks cut from FTA containing human blood (lanes 3–5). The 2.0 mm disks did not give successful amplification in this experiment (lanes 6–8). Added DNA was still amplified in the presence of washed FTA control disks of either size (lanes 9 and 10).

The amplification of human DNA from blood stored on FTA also succeeded with smaller (1.2 mm) but not larger (2.0 mm) disks in two additional experiments (data not shown). However, GenomiPhi did amplify DNA on larger disks of buccal samples, while washed disks did not inhibit amplification of control DNA. It appears that excess DNA template on the larger blood sample disks may inhibit the process.

Conclusions

Human blood DNA preserved on FTA is a suitable sample for whole genome amplification by GenomiPhi. This approach combines the FTA benefits of collecting small original samples and storing them safely and inexpensively at room temperature with the WGA benefits of easily amplifying large enough quantities of DNA for the most demanding applications.

Electrophoresis Gel of Amplified Human Genomic DNA from Blood on FTA



- Lane M: 1 kb DNA ladder
Lane 1: Positive control DNA (no FTA)
Lane 2: Negative control (no DNA or FTA)
Lanes 3-5: Amplifying 1.2 mm sample disks
Lanes 6-8: Amplifying 2.0 mm sample disks
Lane 9: Amplifying 1.2 mm control (unspotted) disk plus positive control DNA
Lane 10: Amplifying 2.0 mm control disk plus positive control DNA

Whatman Quality

Whatman is a global leader in separations technology and is known in the scientific community for providing innovative Life Science products and solutions. Our instinct for simplification accelerates the rate of discovery, reduces costs and saves time. In order to focus on the unique needs of our customers, Whatman is organized into four business development units: Analytical Chemistry, Diagnostics, Genomics & Proteomics and Medical Devices. For more information, visit www.whatman.com.

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